

DEDICATION

To my mother and to the soul of my father

To my dearest wife, my beloved, kid Hamed,

To My sisters and brothers

"To all of them I dedicate this work"

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I would like hereby to thank all my colleagues, without their sincere efforts, this research would have never come to accomplished.

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ABSTRACT

Background: *Tuberculosis* remains a major global health problem. Lymph nodes represent the most common site for extra pulmonary *tuberculosis*. Several techniques have been employed for the diagnosis of extrapulmonary *Tuberculosis* (EPTB) including; conventional Hematoxylin and Eosin stain, the Ziehl-Neelsen(ZN) stain, Immunohistochemistry(IHC) and Polymerase chain reaction (PCR). Each of these techniques has advantages and limitations.

Aim: To evaluate the diagnostic utility of PCR, IHC and ZN stain in detection of *Mycobacterium tuberculosis* in histological sections.

Materials and methods: This is descriptive analytical cross sectional study conducted in Khartoum State during the period from July 2012 to July 2015. In this study 161 lymph nodes tissue biopsies were used in this study. These 161 samples were reinvestigated by H and E. The specific monoclonal anti 38KD was used to detect *mycobacterium tuberculosis* (MTB) in histological section by IHC, and IS6110 sequence was used to detect MTB by PCR. IS6110 PCR assay was performed in comparison with the H&E, ZN stain and IHC.

Results: In this study, 161 enlarged lymph nodes were diagnosed as having lymph node *tuberculosis* by histopathology. The minimum age of study population was 4 years and the maximum was 85 years with a mean age of 51 years. The study population was divided in to two group pediatric 42 (26%) and adult 119 (74%). The male female ratio was 0.89:1.11. The study population subsequently subdivided in to other groups started from >8 years up to 51+. The great majority of the specimens were obtained from cervical lymph node which representing 100 (62%), followed by axillary lymph node representing 17 (11%). The other sites include mediastinal,

mesenteric, inguinal, and submandibular, constituting 10 (6%), 7 (4%), 7 (4%), and 4 (3%) respectively. The study populations were further divided into two groups according to the presences of strong and weak *tuberculosis* histopathological evidences. Accordingly, of the 161 cases, 118 (73.3%) were categorized as having strong evidences and the remaining 43(26.7) were detected with weaker evidences, cases.

In this study, of 161 studied lymph nodes, only 4 (2.5%) were positive with ZN and 157(97.5%) were negative. These 4(2.5%) ZN positive cases were previously found as strong evidence, while the remaining 114 strong evidences cases were negative with ZN stain. Statistically, no significant association between TB histopathologicalevidences and ZN stain, *P-value=0.221*.

IHC monoclonal anti 38-KD was positive in 129 (80%) of cases, the remaining 32 (20%) were negative with IHC. Of the entire 129 IHC positive cases, 100 (62%) cases were identified as strong evidences cases and the remaining 29 (18%) were at weak level. Statistically, IHC stain is significantly associated with TB histopathologicalevidences *P-value = 0.015*.

In this study, of 161 studied lymph nodes, 135 (84%) were positive for IS1160 PCR and the remaining were negative, from the entire 135 (84%) PCR positive cases, 106 cases were previously found as strong evidence and 29 was found weak evidence. Of remaining 26 (16%) PCR negative cases, only 12 specimens were showed strong evidence for TB, and 14 were weak evidence, statistically, PCR is significantly associated with TB histopathologicalevidences, *P-value=0.001*. On the other hand PCR was used as a gold standard for comparing the other variables, accordingly the sensitivity and specificity of histopathology diagnosis & ZN stain were

78.5%, 46.1% and 3.0%, 100% respectively. In contrast the sensitivity and specificity of anti 38KD IHC was 95.5%, 100% respectively.

Conclusion: IHC with monoclonal anti 38KD and PCR with IS6110 oligonucleotides are rapid, sensitive, and specific methods for establishing the diagnosis of *tuberculosis* in histologic specimens. Immunohistochemistry has the advantages over PCR of being robust, quicker, and cheaper, and it can be used in high-endemic countries.

ملخص الدراسة

خلفية: يعتبر مرض الدرن مشكلة صحية عالمية رئيسية. تمثل العقد الليمفاوية الموقع الأكثر شيوعاً لمرض الدرن خارج الرئة، وقد استخدمت العديد من التقنيات لتشخيص مرض الدرن خارج الرئة، بما في ذلك، صبغة الهيماتوكسيلينوالايوسن (H and E) التقليدية، صبغة زيل- نيلسن (ZN)، كيمياء الأنسجة المناعية (IHC) وتفاعل البوليميريز المتسلسل (PCR) للتحقق من وجود مرض الدرن.

الهدف: كان الهدف من هذه الدراسة هو تقييم الفائدة التشخيصية لتفاعل البوليميريز المتسلسل، كيمياء الأنسجة المناعية وصبغة زيل نيلسن في الكشف عن المتفطرة الدرنية في القطاعات النسيجية.

المواد والطرق: هذه دراسة ارتجاعية تحليلية أجريت في ولاية الخرطوم خلال الفترة من يوليو عام 2012 إلى يوليو 2015. في هذه الدراسة 161 عقده ليمفاوية تم استخدامها وأعيد تسخينها بصبغة الهيماتوكسيلينوالايوسن.

في هذه الدراسة، استخدم الجسم المضاد وحيد النسل (KD-38) عن طريق تقنية كيمياء الأنسجة المناعية في الكشف عن المتفطرة الدرنية في المقاطع النسيجية، وايضاً استخدم التسلسل (IS6110) عن طريق تقنية البوليميريز المتسلسل في الكشف عن المتفطرة الدرنية في تلك الانسجة. وتمت مقارنة البوليميريز المتسلسل بالتشخيص النسيجي، صبغة زيل- نيلسن وتقنية كيمياء الانسجة المناعية.

النتائج: في هذه الدراسة الوصفية الارتجاعية كان هنالك عدد 161 مريض مصاب بتضخم العقد الليمفاوية. وتم تشخيصهم عن طريق التشخيص النسيجي على ان لديهم مرض الدرن في العقد الليمفاوية. اظهرت التحليل الاحصائية أن أقل أعمار المرضى الذين شملتهم الدراسة كان 4 سنوات واعلاها 80 سنة بمتوسط عمر 51 سنة. تم تقسيم المرضى الى مجموعتين، أطفال وكان عددهم 42 (26%) وكبارو كان عددهم 119 (74%)، وكانت نسبة الذكور للاناث 0.89 : 1.11. لاحقاً تم تقسيم المرضى الى مجموعات اخري تبتداء بأقل من عمر 8

سنوات وتنتهي بأكثر من 51 سنة. في هذه الدراسة معظم العينات تم الحصول عليها من العقد الليمفاوية السطحية ومثلت 100 (42%) عقدة ليمفاوية، تبعثها العقد الليمفاوية الأبطية ومثلت 17 (11%)، العقد الليمفاوية الأخرى مثل العقدة الليمفاوية المنصفية، العقد الليمفاوية المسارية، العقد الليمفاوية الأربية والعقد الليمفاوية تحت الفك السفلي مثلت 10 (6%)، 7 (4%)، 4 (3%) على التوالي أيضاً تم تقسيم المرضى لاحقاً إلى مجموعتين اعتماداً على وجود الدلائل القوية والضعيفة لمرض الدرن، واعتماداً على ذلك، من 161 حالة كان هناك 118 (73.3%) حالة أعطت دلائل قوية لوجود مرض الدرن، والمتبقية 43 (26.7%) ذات دلائل ضعيفة لمرض الدرن.

في هذه الدراسة، من عدد 161 عقدة ليمفاوية تمت دراستها بواسطة صبغة زيل-نيلسن وكان هناك 4 (2.5) عقدة ليمفاوية أعطت نتائج موجبة لمرض الدرن، والمتبقية 157 (97.5%) أعطت نتائج سالبة. هذه الأربعة (2.5%) حالات الموجبة لصبغة زيل-نيلسن كانت في السابق ذات دلائل قوية لوجود مرض الدرن، والمتبقية 114 من الحالات القوية الدلائل لمرض الدرن أعطت نتائج سالبة لصبغة زيل-نيلسن.

كانت كيمياء الأنسجة المناعية باستخدام الجسم المضاد وحيد النسب (KD-38) موجبة في 129 (80%) حالة، والمتبقية 32 (20%) حالة كانت سالبة. ومن 129 حالة موجبة، كانت هناك 100 (62%) حالة ذات أدلة قوية لوجود المرض و 29 (18%) ذات أدلة ضعيفة لوجود المرض. إحصائياً تقنية كيمياء الأنسجة المناعية لها ارتباط كبير مع دلائل مرض الدرن، حيث كانت القيمة الاحتمالية = 0.015.

في هذه الدراسة أيضاً، من 161 عقدة لمفاوية تمت دراستها، كانت هناك 135 (84%) حالة موجبة لتقنية المبوليميريز المتسلسل والمتبقية كانت سالبة. ومن 135 حالة موجبة، كانت هناك 106 حالة وجدت سابقاً ذات أدلة قوية لوجود مرض الدرن و 29 حالة ذات أدلة ضعيفة. إحصائياً تقنية المبوليميريز المتسلسل لديها ارتباط كبير مع دلائل مرض الدرن، حيث كانت القيمة الاحتمالية = 0.001.

من ناحية اخرى تم استخدام البوليميرز المتسلسل كمعيار ذهبي لقياس المتغيرات الأخرى. وعليه كانت حساسية وخصوصية صبغة زيل-نيلسن وصبغة الهيماتوكسولينوالايوسن 3.0%، 100% و 78.5%، 46.1% على التوالي، وفي المقابل حساسية وخصوصية كيمياء الانسجة المناعية 95.5%، 100%.

الخاتمة: تقنية كيمياء الانسجة المناعية بأستخدام الجسم المضاد وحيد النسل (38-KD) وتقنية البوليميرز المتسلسل بأستخدام التسلسل (IS6110) هي طرق سريعة، حساسة، وذات خصوصية في تشخيص المتقطره السلية في العينات النسيجية. كيمياء الانسجة المناعية لديها مزايا أكثر من البوليميرز المتسلسل، كونها قوية، أسرع، وأرخص، ويمكن استخدامها في البلدان المستوطنة.

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Publications

- 1- **IhabHamedNourein,** HussainGadelkarim Ahmed, AbuobiedaBallaAbusharib, MaysaBadawiElmubasher, Sara AbulGasimSeif el din, FawazAlshammari. *"Diagnostic Utility of ZN, IH and PCR in Detection of Tuberculous Lymphadenopathy: A Retrospective Study from Sudan."* *International Journal of Science and Research (IJSR)* 3, no. 7, (2014): 1402-1406. www.ijsr.net/archive/v3i7/MTcwNzE0MDM=.pdf.
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- 3- **IhabHamedNourein,** HussainGadelkarim Ahmed, Hassan Elsiddig Hassan, Mohammed AyedHuneif, Abdelrahman Mohamed Abdelrahman, AbuobiedaBallaAbusharib. *"Monoclonal Anti 38-KD Immunohistochemistry: A Novel Method for Improving the Diagnosis of Pediatric Tuberculous Lymphadenitis."* *International Journal of Science and Research (IJSR)* 3, no. 10. (2014): 88-91. www.ijsr.net/v3i10.php.

List of abbreviations

Abbreviations	Title
AC	Adsorption column
ADIS	Acquired Immunodeficiency Syndrome
AFB	Acid fast bacilli
BAL	Bronchial Aspiration Lavage
BB	Bronchial Brushings
BCG	Bacilli Calmette–Guérin
BW	Bronchial Washings
CD	Cell Differentiation
CT	Computerized tomography
DAB	Diaminobenzidine
DNA	Deoxyribo nucleic acid
DPX	Distrene, Plasticiser, Xylene
EB	Elution buffer
EPTB	Extrapulmonary <i>tuberculosis</i>
ESAT-6	Early-secreted antigenic target-6
FFPE	Formalin- fixed, paraffin- embedded
FISH	Fluorescence in situ hybridization
FNAC	Fine needle aspiration cytology
H and E	Hematoxylin and Eosin
HIV	Human Immunodeficiency Virus

HRP	Horse reddish peroxidase
IGRAs	Interferon-gamma release assays
IHC	Immunohistochemistry
IL	Interlukin
ISH	In situ hybridization
KD	Kilo Dalton
LJ	Lowenstein–Jensen
LN	Lymph node
LNTB	Lymph node <i>tuberculosis</i>
MRI	Magnetic resonance imaging
MTB	<i>Mycobacterium tuberculosis</i>
MTC	<i>Mycobacterium tuberculosis</i> complex
NAA	Nucleic acid amplification
NK	Natural Killer
NTM	Non-tuberculous mycobacteria
PBS	Phosphate buffer Saline
PBS	Post bronchoscopy sputum collection
PCR	Polymerase chain reaction
PPD	Purified Protein Derivative
PTB	Pulmonary <i>tuberculosis</i>
RNA	Ribo nucleic acid
RPM	Round per minute
SI	Sputum Induction
SPSS	Statistical Package for Social Sciences
TB	<i>Tuberculosis</i>
TBB	Transbronchial biopsy
TBE	Tris/Borate/EDTA

TBL	Tuberculous lymphadenitis
TNF α	Tumor Necrosis Factor α
TST	Tuberculin skin test
UV	Ultraviolet
WHO	World Health Organization
ZN	Ziehl-Neelsen